Glycerol Stock of Anaerobic AMB-1

Short-Term Protocol:

We are supplying a liquid culture (1.7 ul) of wild-type AMB-1 (AK30) in a microcentrifuge tube.

As a general guide to how this tube would be propagated for a short-term experiment we would suggest:

1) Inoculate cells from 1.7 ul tube into a 10 ml culture (1:100 dilution) with MG liquid media (see recipe below)
2) Grow culture at 4% O_2, 30◦C overnight

Long-Term Protocol:

As a general guide to how this tube would be propagated for longer-term experimentation we would suggest:

1) Inoculate cells from 1.7 ul tube into a 50 ml sterile falcon tube (1:100 dilution) with MG liquid media + Vitamins and Iron (see recipe below)
2) Grow 50 ml culture for 2 days at 30◦C (no need for 4% O_2 as depletion in full falcon tube is sufficient)
3) Spin down cells (8000 X g at room temperature for 10 minutes)
4) Create glycerol stock (30% glycerol final)
5) Then, one would streak out cells onto MG Agar plates
6) Plates require 4% O_2, 30◦C for 5-7 days to form single colonies
7) Pick colonies into 1.7 ml tubes with MG media (as shipped example is a representative of)
8) - 90% total volume milliQ water